MedCrave
Step into the World of Research
Horticulture International Journal

Research Article
Open Access

Natural coloring red beetroot under effects of gamma radiation

Volume 5 Issue 3 - 2021

Marcia Nalesso Costa Harder,[1,2] Ian Harder Formaggio,[2] Larissa Nalesso Costa Harder,[1] Débora Cristina Maciel de Lemos Bovi,[2] Paula Bergamin Arthur,[1] Valter Arthur[1,3]

[1]Nuclear and Energy Research Institute, São Paulo, Brazil
[2]Technology College of Piracicaba, Piracicaba, São Paulo, Brazil
[3]Radiobiology and Environment, Center of Nuclear Energy in Agriculture, Piracicaba, São Paulo, Brazil

Correspondence: Valter Arthur, Center of Nuclear Energy in Agriculture, Av. Centenário, 303, Piracicaba, São Paulo, Brazil, Tel 5519 34294665, Email arthr@cena.usp.br, marciaharder@fatec.sp.gov.br

Received: May 30, 2021 | **Published:** June 15, 2021

Abstract

As color is an important attribute related to the visual appeal and quality of food products, the reduction of pigment losses during food processing is a basic concern for the industry. Based on this, the objective of this work was to evaluate the resistance of betalain from beet against the use of gamma radiation. Analyzes were carried out on pigments belonging to the betalain class on beets exposed to different doses of gamma radiation (Co60) using doses of 5, 10 and 15 kGy on beets, in addition to anon-irradiated sample for control. Color parameters were evaluated using the CIE L, a*, b * methodology, as well as the quantification of betalain in the samples. By of results the color parameters were affected in until 50% in relation the control. And the quantity betalain activity in the sample irradiated with doses of gamma radiation was more less 65% in relation the control. Therefore, it can be concluded that there is a potential resistance of the betalain pigment in the face of high doses of radiation, which returns interest to red beetroot dyes since most pigments are fragile to thermal treatments or with ionizing radiation.

Keywords: *Beta vulgaris* L., betalain, coloring resistance, food radiation, ionizing radiation

Introduction

Color is associated with many aspects of our lives, influencing our decisions, especially those involving food. Food appearance, safety, acceptability and sensory characteristics are all affected by color. Although these effects are associations inherent to psychological characteristics, they interfere with the choice of products. Among the strategies of the industries to enrich and favor the product that is processed, the dye has a stimulating effect on the consumer's appetite.[1]

Natural dyes are understood as all organic compounds that have the ability to selectively absorb light acquiring intense coloring, consequently giving it to the bodies which its adheres. Chemically, dyes are only substances capable of coloring irreversibly a matrix.[2]

In recent years, consumers of colored products have increased their rejection about using artificial colors. At the same time, mainly the coloring of foods and cosmetics using natural sources containing different classes of pigments has gained importance in the industry.[3]

These authors also report that the predominant colors of plants result from classes of pigments of the type chlorophyll, carotenoid and flavonoid, while the contribution of other pigments from betalains, melanins, the many flavonoids and others are considered insignificant when considered globally. Pigments produce colors and are present in all organisms in the world, plants are the largest producers of these pigments found in leaves, fruits, vegetables, flowers, as well as in animals, bacteria and fungi.

According to Moura et al.[4] the beet (*Beta vulgaris* L.) belongs to the Cheno podiaceae family, being an easy-to-grow vegetable garden with a vegetative cycle of three to four months. Red beet contains dyes that have been arousing a particular interest as an additive in the food industry since its use is not restricted worldwide by FAO/WHO.

In the beet, the presence of a red pigment with polar characteristics is evidenced, the substance betanine, a pigment that is belonging to the group of betalains and which corresponds to between 75% and 95% of the pigments. Betalains are stable at pH 4 and 5; and reasonably stable at pH 5 to 7 and unstable in the presence of light and air, in addition to these factors, water and oxygen activity affect pigment stability.[5,6]

Betalains are pigments that contain nitrogen and are soluble in water.[7] Betalain pigments can be described as a 1,2,4,7,7-pentasubstituted 1,7-diazoheptamethinesystem[8] (Figure 1). They can be divided into two structural groups: yellow beta xanthines and red-violet beta-cyanines depending on the substituents R1-N-R2.[9]

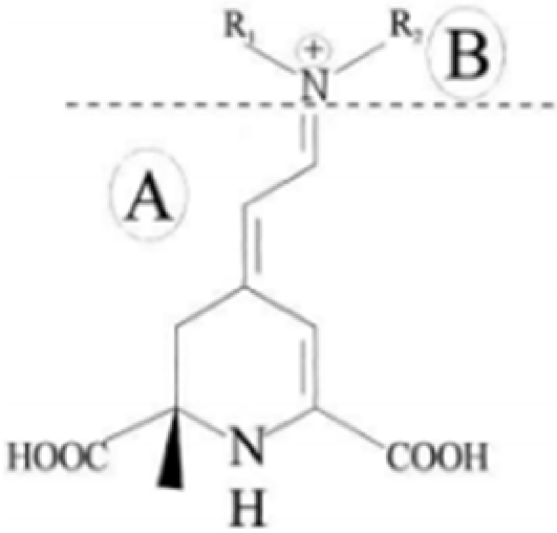

Figure 1 General formula of betalains. (A) betalanic acid is present in all betalain molecules. (B) the structure Represents betacyanine or betaxanthine depending on the substituents R1 and R2 (Horst, 2005).

Betalains represent a group of natural compounds that are particularly fascinating due to their structural and chemotaxonomic properties. Betalains are mainly accumulated or stored in flowering and inflorescence plants, petiole, bract, fruit, seeds, stem, leaf and root.[10]

And within the context of the use of natural dyes from plants that are currently gaining popularity for the use as natural dyes in the food industry, betalain from red beetroot presents itself as one of the most widely used food dyes. Despite this, betalains are not so well studied in comparison with other natural pigments, such as anthocyanins, carotenoids and chlorophylls,[11] including the stability of this dye to the different situations present during the phases of industrial processing.

For Horst,[9] when betalains are used as dyes in foods, color stability is the main interest. There are several factors that affect the stability of these pigments during the preparation, processing and storage of food such as light, pH, temperature, metal ions and others.

The use of ionizing radiation in the food industry has been widespread as the benefits obtained have already become evident, especially with regard to food security, corresponding to an advance in production techniques. Safety in the use of the technique and the longevity provided by this technology to food products have already been demonstrated.

However, the stability of food dyes, especially natural ones, tends to be sensitive to the use of this technology. But according to Consentino,[12] there are few records on the stability of natural dyes in the face of the ionizing source irradiation process. However, to the author, it is possible to find references regarding the behavior of the dyes against radiation in the visible spectrum, temperature and presence of oxygen, and the combination of several physical-chemical factors acts on the structure of the dyes generating instabilities of orders, causing damage to the quality of the dye.

Justifying the restriction of works related to the stability of natural dyes when using ionizing radiation, this study aimed to evaluate the resistance of betalain from red beet submitted to the source of gamma radiation by Cobalt-60.

Material and methods

The red beetroot was purchased from local businesses in the city of Piracicaba in the state of São Paulo. After being acquired, the samples were taken to the Radiobiology and Environment laboratory of the Center for Nuclear Energy in Agriculture (CENA / USP), where they were cleaned and prepared to receive the treatments.

After making the trays, the irradiation treatments were carried out at the Center for Nuclear Energy in Agriculture (CENA / USP) also located in the city of Piracicaba. The prepared red beetroot samples were irradiated in a Cobalt-60 radiator, type Gammabeam650, with doses of 0 (control); 0; 5; 10 and 15 kGy in chronic rates, under a rate of dose of 0.259 $kGy.hour^{-1}$, and each treatment consisted of 4 repetitions and stored at 2ºC to 6oC and 80% RH.

Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) and the free radical signal was mensured with Bruker EMS 104EPR Analyzer. The actual dose was within 0.02 of the target dose. Samples was turned 360continuously during the irradiation process to achieve uniform target doses and the non irradiated control was placed outside the irradiation chamber to have the same environmental temperature effect with the irradiating sample.

After the irradiation, it was evaluated the physico-chemical analyses.

Physico-chemical analysis

Color

The colorimeter Minolta CR-200 b was used, previously calibrated in White according to pre-determined standards, according to.[13]

Three values of chroma were evaluated: a*, b* and L. The value a* characterizes thecolor from the red (+a*) to the green (-a*); the value b* indicates the color from the yellow (+b*) to the blue (-b*). The value L determines the light ranging from white (L=100) to black (L=0). The chroma is the ratio between a* and b*, where the real color can be obtained. Hue-Angle is the angle between a* and b*, indicating the color saturation of the analyzed object.[14,15]

To estimate chroma value, the following formula was adopted (1) and to estimate the Hue-Angle, formula (2).[16]

$$C=\sqrt{(a2+b2)} \quad (1)$$

$$H^o=arcpg\ b^*/a^* \quad (2)$$

Betalain analysis

The extraction with solvent method (distilled water) was used to extract the pigments from the reed beetroot, being 0.087 g of red beetroot previously crushed and mixed with 30ml of distilled water in a beaker and placed in a water bath with agitation at 70° C for five minutes. Soon after, they are centrifuged at 12,000 rpm (revolutions per minute) for fifteen minutes.

The pigment absorbance was determined at a wavelength of 538 nm in a spectrophotometer. The betacyanin content was calculated according to Lim et al.[17] and adapted from Bovi et al.[18] following the formula below (3):

Beta cyanin content (mg / 100 g fresh weight) = $A538\ (MW)\ V\ (DF)\ \varepsilon\ LW \times 100$ (3)

In which:

A538 = absorbance at 538 nm (E max); L (bucket thickness) = 1.0 cm; DF = dilution factor; V = volume of extract (ml), W = fresh weight of red beetroot in the extract (g). For betalain the molar absorptivity ε = 6.5 × 104 L / mol cm in H2O; and MW (molecular weight) = 550.

Statistical analysis

The experimental design was complete randomized with three replications. The results were analyzed according to(ANOVA) using the F test, and mean comparisons were tested based on Tukey´s test ($p<0,05$) using SAS.[19]

Results

From the results obtained, Table 1 shows the mean values obtained in the color analyzes for L, a * and b * of the outer part (peel) of the red beetroot and Table 2 shows the mean values obtained from the color analysis of the inner part of the red beetroot (Table 1 & Table 2).

Table 3 shows the mean values obtained in the color analyzes of the Chroma and Hue Angle.

The betalain values obtained from the pigment absorbance in a spectrophotometer at a wavelength of 538 nm are shown in Table 4.

Citation: Harder MNC, Formaggio IH, Harder LNC, et al. Natural coloring red beetroot under effects of gamma radiation. *Horticult Int J.* 2021;5(3):129–132. DOI: 10.15406/hij.2021.05.00218

Table 1 Mean values and standard deviation of the color analysis of the external part of the red beet root

Treatments	Doses (kGy)	L	a*	b*
T0 (Control)	0.0	43.03±1.45*a**	5.38±0.22a	14.79±1.03a
T1	5.0	33.32±1.09a	5.13±0.15a	8.80±1.00b
T2	10.0	33.14±0.99a	5.94±0.27a	8.93±0.88b
T3	15.0	36.90±1.25a	10.32±1.09b	10.46±1.21b

*Media ± standard deviation

**Equal letter values in column do not differ statistically by Tukey's test at 5% significance level

Table 2 Mean values and standard deviation of the color analysis of the inner part of the red beetroot

Treatments	Doses (kGy)	L	a*	b*
T0 (Control)	0.0	29.57±1.06*a**	36.90±1.09a	18.15±1.65a
T1	5.0	22.02±1.12a	26.83±0.99b	15.86±1.03b
T2	10.0	22.10±1.10a	25.50±0.87b	13.65±0.99c
T3	15.0	18.43±0.87a	23.98±0.78b	9.55±0.79d

*Media ± standard deviation

**Equal letter values in column do not differ statistically by Tukey's test at 5% significance level

Table 3 Mean values and standard deviation of the of the color parameters Chroma and Hue-Angle of red beetroot

Treatments	Doses (kGy)	Chroma	Hue-Angle
T0 (Control)	0.0	41.12±1,20*a**	0.45±0.01a
T1	5.0	37.26±1.17b	0.76±0.21a
T2	10.0	28.92±1.20c	0.49±0.32a
T3	15.0	25.81±1.35d	0.37±0.11a

*Media ± standard deviation

**Equal letter values in column do not differ

Table 4 Mean values and standard deviation of the of the betalain quantity of red beetroot

Treatments	Doses (kGy)	Betalain (mg/100g beetroot)
T0 (Control)	0.0	31.58±0.66*a*
T1	5.0	22.77±1.32b
T2	10.0	21.48±3.30b
T3	15.0	20.09±3,96b

*Media ± standard deviation

**Equal letter values in column do not differ statistically by Tukey's test at 5% significance level

Discussion

From the data presented in Tables 1 and 2, it can be seen that there was no significant change in parameter L. However, there were significant changes for parameters a* and b*, as the radiation dose increased. For parameter a* and b*, the changes were significant from the application of 5.0kGy.

The data obtained agree with the results of Chung et al.[20] with which they state that gamma irradiation at 5.0 kGy reduced the main dyes of red beetroot by 94%. However, they disagree with the authors when they state that the reddish color disappeared completely with doses above 10.0 kGy.

For Chung et al.[20] the explanation for the color removal is probably due to the effects of ethanol gamma radiolysis. For the authors, the details of the mechanism of destruction of the chromophore by gamma rays have not been fully explained, but the results of their study suggest that the free radicals produced are capable to destroy the chromophore group in all (iso) bethanines, which resulted in bleaching the substrate solution.

Hernandes et al.[21] who used gamma radiation in minimally processed red beetroots to prolong shelf life, observed that doses 1.5 kGy do not sensorially alter the appearance of the product for evaluators in up to 20 days of storage by sensory analysis, what corroborates with this work the possibility of storing the product, since doses above the dose used by the authors were used.

The data obtained in this work also agree with the data obtained by Nunes et al.[22] since he found no statistically significant difference between samples of red beetroot irradiated with up to 4.0 kGy, finding difference only on parameter b* in the treatment with 4.0 kGy.

It is noted that the doses affected the external/internal color of the red beetroot, making it darker, since the parameter L at doses 5.0, 10.0 and 15.0 kGy decreased in relation to the control sample (T0).

For Table 3, Prieto-Santiago et al.[23] described that the two chromatic parameters a* and chromaare proposed as the best descriptors for the betalain concentrations.

For Kim et al.[24] in Hunter's color value, lightness (L), redness (a*) and yellowness (b*) occurred a decreased with increment of irradiation dose, agreeing the results presented in Tables 1–3.

On Table 4 it is possible to see that there was a significant decrease in the absorbance in the sample irradiated proportional the increase of gamma radiation doses when compared with the control sample. Regarding the control sample was had major mean values in absorbance. This represents a whitening of the pigment in the gamma radiation doses.

The results are agreed to Kim et al.[24] who showed that there was no differencein the amount of betalain extracted by irradiation. However, the values obtained is disagree with the authors, who claim that their results exhibit high values of optical density at an irradiation dose of 5.0 kGy.

The medium values obtained on the amount of betalain in this study is in agree with the values obtained by Bovi et al. (2019) who presented a value of 31.42mg/100g of fresh red beetroots and Vitti et al.[25] who presented a value of 35mg/100g of red beetroots.

Although, considering natural colorant and textural quality like in this work, for Kim et al.[24] the gamma irradiation above 10.0 kGy was an undesirable technique for red beetroots, but how can see in this work was the radiation dose of 5.0 kGy for red beet roots, but it is possible to see that the quantity betalain activity in these doses was more less 65%.

Another point observed was the resistance of this pigment to high doses of radiation, what returns interest to beet dyes since most pigments are fragile to thermal treatments or with ionizing radiation. Therefore, it is worth noting that red beetroot as a pigmenting agent and color supplier maintains some resistance to the use of gamma radiation.

Conclusion

By the results it can be observed that according with the increase of radiation doses, the colorparameters were affected in until 50% in relation the control. And the quantity betalain activity in the sample irradiated with doses of gamma radiation was more less 65%in relation the control.

Funding

None.

Acknowledgments

None.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Souza, RM. Corantesnatura is alimentícios e seusbenefícios à saúde. Rio de Janeiro. Centro Universitário Estadual da Zona Oeste. 2012;65.
2. Franco CFO, Harder MNC, Arthur V, et al. Quebra de dormência e influêncian osteores de bixin aemsementes de urucumsubmetidas à radiaçãogama. *Tecn Ciênc Agrop*. 2013;(7):13–18.
3. Schiozer AL, Barata LES. Estabilidade de corantes e pigmentos de origem vegetal. *Rev Fitos*. 2012;3:6–24.
4. Moura LL, Cascon SC, Guimarâes ISS. Corantes de beterraba e suaaplicaçãoe malimentos. Rio de Janeiro. Embrapa. 1989;4.
5. Dias MV, Guimarães PI, Merçon F. Corantesnaturais: extração e empregocomo indicadores depH.Quím Nova Escola. 2003;17:27–31.
6. Cuchinski AS, Caetano J, Dragunski DC. Extraction of the dye of beet (*Beta vulgaris*) for useas indicated in acid-base. *Ecl Quím*. 2010;35:17–23.
7. Strack, D, Vogt T, Schliemann W. Recent advances in betalain research. *Phytochem*. 2003;62:247–269.
8. Von Elbe JH, Schwartz SJ. Colorants. food chemistry. Ed. By Fennema, O.R., 3 ed. New York, Marcel Dekker, Inc. 1996;651–722.
9. Horst BL. Estudo da estabilidade do corante natural betalaína microen capsulado com matrizpolimérica de quitosana/alginato. Florianópolis. *UFSC*. 2005;46.
10. Coy-Berrera E. Analysis of betalains (betacyanins and betaxanthins). *R Adv NatProd Anal*. 2020;593–619.
11. Choo WS.. Betalains: Application in functional foods. bioactive molecules in food, reference series in phytoc hemistry. Ed. by Mérillon JM. And Ramawat K. 2007;1–28.
12. Consentino HM. Efeito da radiaçãoion izantee mcorantes natura is de usoalimentício. São Paulo.IPEN. 2005;149p.
13. Bible BB, Singha S. Canopy position influences cielab coordinates of peach color. *Hort sci*. 1997;28:992–993.
14. Harder MNC. Efeito do urucum (*Bixaorellana* L.) naalteração de característica de ovos de galinha poedeira. Piracicaba. Universidade de São Paulo. 2005;74.
15. Harder MNC, Canniatti-Brazaca SG, Arthur V. Quantitative evaluation by a digital colorimeter of the color of the egg of laying hens fed with annatto (*Bixaorellana*). *Rev Port Ciênc Vet*. 2007;102:339–342.
16. Estevez M, Cava R. Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pate. *Meat Sci*. 2004;68:551–558.
17. Lim SD, Yusof YA, Chin NL, et al. Effect of extraction parameters on the yield of beta cyanins from pitaya fruit (*Hylocereus polyrhizus*) pulps. *J Food Agric Environ*. 2011;9:158–162.
18. Bovi DCML, Correia AFK, Gutierrez EMR, et al. Determinaçãodos teores de betalaína e composição centesimal de beterraba in natura e tipo chips. *Braz J Food Res*. 2019;10:80–92.
19. SAS Institute. SAS/QC software: usage and reference (version 9.2). Cary, NC, 2005.
20. Chung BY, Lee SS, Lee EM, et al. Removing undesirable color and boosting biological activity in red beet extracts using gamma irradiation. *In 2012 International Conference on Biomedical Engineering and Biotechnology*. IEEE. 2012;489-491.
21. Hernandes NK, Coneglian RCC, Godoy RLO, et al. Sensory acceptance testes of red beet *(Beta vulgaris ssp. vulgaris* L.), cv. Early Wonder, minimally processed and irradiated. *Ciênc Tecn Alim*. 2007;27:64–68.
22. Nunes TCF, Rogovschi VD, Fabbri ADT, et al. Colorimetric evaluation of irradiated red beetroots. International Nuclear Atlantic Conference. *ABEN*. 2013;978–985.
23. Prieto-Santiago V, Cavia MM, Alonso-Torre SR, et al. Relationship between color and betalain content in different thermally treated beetroot products. *J Food Sci Techn*. 2020;57:3305–3313.
24. Kim KH, Lee SA, Yook HS. Effects of gamma irradiation on physicochemical properties ofred beet and stability of betalain in the red beet (*Beta vulgaris* L.). *J Kor Soc Food Sci Nut*. 2007;36:453–457.
25. Vitti MCD, Kluge RA, Yamamotto LK, et al. Comportamen to de beterr abasminimamente process adasem diferent esespessuras de corte. *Hortic Bras*. 2003;21:623–626.